Studies on Polymeric Nanoparticles Loaded With Synthetic Pyrethroid and Essential Oil from Plants against Malaria Vector

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INTRODUCTION

Vector borne diseases are the major health problem all over the world and causing millions of deaths every year and mosquito is still the major vector among all other vectors of diseases. Mosquitoes are responsible for spreading various human dreaded diseases like malaria, lymphatic filariasis, dengue, chikungunya, yellow fever etc. Mosquitoes have approximately 3500 species present in tropical and subtropical regions of the world **(Naseem *et al.*, 2016).** *Anopheles stephensi* is a primary vector of urban malaria and disseminated various species of *Plasmodium* in India **(Talukdar *et al.*, 2017).** It has estimated that half of the world's population is at risk of malaria and 243 million cases led to nearly 8,63,000 deaths in 2008 **(WHO, 2009)** and 216 million cases of malaria in 91 countries were reported with an increase of 5 million cases over 2015 **(WHO, 2018)**. Malaria infection during pregnancy is a significant public health problem with substantial risks for the pregnant woman, her fetus, and the newborn child and associated problems like maternal illness and low birth weight is mostly the result of *Plasmodium falciparum* infection and predominantly occurred in Asian countries **(WHO, 2018)**. Therefore, the control of malaria vector i.e., *Anopheles* mosquito is essential to overcome the menace of malaria.

Synthetic pesticides are effective, fast reacting, easily available and easy to use. But the indiscriminate use of synthetic insecticides showed an alarming increase of physiological resistance in the vectors, toxicity to non-target organism, hazardous to environment and some of insecticides act as carcinogenic agents are noteworthy **(Subramaniam *et al.*, 2012)**. Plant derivatives being the potent eco-friendly, target specific, easily available and cost effective are may be the alternative to control the ever increasing population of mosquito vectors. Phytochemicals of plant essential oils have different promising insecticidal properties *viz*. ovicidal **(Mya *et al.*, 2017),** larvicidal **(Sogan *et al.*, 2018),** adulticidal **(Prabhavathi *et al.*, 2016)** and repellent activities **(Auysawasdi *et al.*, 2016)** against mosquitoes. In this context, the essential oils and their constituents have received much attention as potentially useful

bioactive compounds against insects. The complex and variable mixtures of bioactive compounds with different modes of action, offered by plants, may lessen the chance of resistance in mosquito population. In view of this, the search of essential oil based combinations that are expected to supplement synthetic pesticide to enhance their potency is required. Joint action of synthetic and natural pesticides is may be the solution of the problem. This approach is the double edged weapon as this would minimize the amount of both the ingredients and would make the application more effective, economical and comparatively less hazardous to the environment.

Currently application of nanotechnology in insect pest management is of the great interest which reduces the particle size of insecticide up to nano scale and enhances their binding capacity to target site. Presently, the technocrats draw their attention towards development of metallic nanopesticides like platinum (Pt), silver (Ag), gold (Au) and copper (Cu) etc. But these metals have toxic effects on the environment, non-target organism and human health **(Kitherian, 2016).** The phyto-nanoparticles are eco-friendly alternative with low cost and easy to obtain as compared to metallic nanoparticles. The polymer encapsulated nanoparticles loaded with plant derivative are advantageous as they may efficiently take by target organisms and perform effectively as compared to macro and non-encapsulated form. However, proper attention has not been paid on synergistic phenomenon between the diverse components of essential oils and synthetic pesticides in nanoformulation, although they may provide better bioefficacy response than the individual components.

In view of the above, the present study is planned to evaluate the bioefficacy of synthetic pyrethroids and plant derived essential oils alone and in combinations with the aim do develop their polymeric nanoformulation against *An. stephensi* .

REVIEW OF LITERATURE

The literature which acquired considerable attention owing the intense range properties of synthetic pesticides, plant derivatives joint properties' and nanoparticles and their bio-efficacy evaluation against common mosquito vectors is summarized under.

➢ SYNTHETIC PESTICIDES AND MOSQUITO CONTROL

Corbel *et al.* (2003) reported the pyrethroid insecticides tested permethrin and propoxur as compounds used for mosquito control against larvicidal activity of *Culex quinquefasciatus*. **Ansari *et al.* (2004)** studied the synthetic pesticide pirimiphos-methyl against malarial vector *Anopheles stephensi*, *Anopheles culicifacies* and *Culex quinquefasciatus*. **Etang *et al.* (2004)** tested the malaria control synthetic pyrethroid DDT against *Anopheles gambiae*. **Bansal and Singh (2006)** evaluated the three synthetic pyrethroids *viz* cypermethrin, lambda cyhalothrin and cyfluthrin larvae against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. **Mohan *et al.* (2008)** studied the bio-efficacy of synthetic pesticide chlorpyriphos and temephos against third instar larvae of *Anopheles stephensi* and *Culex quinquefasciatus*. **Tikar *et al.* (2011)** has been used the DDT, malathion, deltamethrin and fenthion, temephos, chlorpyriphos and malathion at larvicidal and adulticidal susceptibility studies against *Anopheles stephensi* and *Anopheles subpictus*. **Uragayala *et al.* (2015)** reported the larvicidal activity of three neonicotinoids (imidacloprid, thiacloprid and thiamethoxam) against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. **Forstinus *et al.* (2017)** reported the use of phytochemicals as possible alternative to chemical insecticides (pyrethroids and organophosphates) against malaria vector control.

➢ BOTANICALS AND MOSQUITO CONTROL

Aidaross *et al.* (2005) evaluated the larvicidal activity of essential oils and extracts of *Ocimum basilicum* and *Cymbopogon citratus* against *Culex quinquefasciatus*. **Amer and Melhorn (2006)** studied the larvicidal effects of 41 plants essential oils out of these 13 oils (camphor, thyme, amyris, lemon,

cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum and sandalwood) have been induced 100% mortality after 24 h, or even after shorter periods against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. **Batabyal *et al*. (2009)** studied the larvicidal toxicity of *Azadirachta indica* fruits and seed extracts of bitter ground (*Momordica charantia*) and castor (*Ricinus communis*) against filarial vector, *Culex quinquefasciatus*. **Manimaran *et al.* (2012)** reported the larvicidal activity and knockdown effect of 25 essential oils and out of them 8 oils *viz*. calamus, cinnamon, citronella, clove, eucalyptus, lemon, mentha and orange exhibited 100% larvicidal activity at 1000 ppm against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. **Ojha and Pattabhiramaiah (2013)** evaluated the phytochemicals composition and larvicidal activity of *Jatropha curcas* seed oil against *Aedes aegypti*. **Ghosh *et al.* (2013)** studied the larvicidal activity of basil oil against *Aedes aegypti*. **Akono *et al.* (2015)** reported the larvicidal activity of certain essential oils from the pericarps of ripe fruits of *Citrus aurantifolia, C. limon, C. sinensis and C. reticulata* against *Anopheles gambiae*. **El-Akhal *et al.* (2015)** performed the larvicidal activity of essential oils of *Citrus sinensis* and *Citrus aurantium* against malaria vector, *Anopheles labranchiae*. **Tyagi *et al.* (2017)** studied larvicidal activity of essentials oils derived from flowering parts of *Jasminum officinale*, clove oil derived from flower bud, stem, leaf against two mosquito species *Aedes aegypti* and *Anopheles stephensi*. **Iqbal *et al.* (2018)** tested indigenous plants extracts of *Cinnamomum tamala, Aloe vera, Datura alba, Allium sativum, A. cepa, Zingiber officinale* and *Ocimum basilicum* for their larvicidal efficacy against *Culex quinquefasciatus*.

- **JOINT ACTION OF BOTANICAL AND SYNTHETIC PESTICIDES**

Harve and kamath (2004) used plant extracts (*Murraya koengii*, *Coriandrum sativum*, *Ferula asafetida* and *Trigonella foenum graceum*) synthetic pesticides (fenthion and temphos) combination to evaluate their larvicidal activity against *Aedes aegypti*. **Shaalan *et al.* (2005**) reported the synergistic larvicidal efficacy of seeds of *Khaya senegalensis, Daucus carota* extracts with synthetic insecticides fenitrothion, lambda-cyhalothrin and methoprene against *Aedes aegypti* and *Culex annulirostris*. **Mohan *et al*. (2007)**

studied the combinatorial larvicidal action of plant root extract *Solanum xanthocarpum* and synthetic pyrethroid, cypermethrin against malaria vector, *Anopheles stephensi*. **Mohan *et al.* (2010)** studied the combinatorial larvicidal action of *Solanum xanthocarpum* petroleum ether plant roots extract and synthetic insecticide fenthion against *Culex quinquefasciatus*. **Darriet and Chandre (2013)** evaluated the combinations containing pyrethroid (deltamethrin and piperonyl butoxide) synergist and six neonicotinoid insecticides acetamiprid, nitenpyram, thiamethoxam, clothianidin, imidacloprid and thiacloprid against *Aedes aegypti* and *Anopheles gambiae*. **Mohan *et al.* (2013)** studied the synergistic bioefficacy of different concentrations of temphos and fenthion with *solanum xanthocarpum* extracts against *Anopheline* larvae. **Bhan *et al.* (2015**) has been evaluated the *Cuscuta reflexa* petroleum ether extract and its synergistic activity with temephos against mosquito larvae, *Anopheles stephensi* and *Culex quinquefasciatus*. **Shrankhla *et al.* (2015)** studied the larvicidal activity of different combination of synthetic insecticide, temephos with leaf extract of *Pseudocalymma alliaceum* against *Anopheles stephensi* and *Culex quinquefasciatus*. **Gross *et al.* (2017)** reported the synergistic properties of 35 essential oils and piperonyl butoxide to enhance the toxicity of permethrin against the resistant strain of *Aedes aegypti* and *Anopheles gambiae*. **Gnankine and Bassole (2017)** reported the synergistic toxicity of plant essential oils and synthetic pyrethroid as ovicidal, larvicidal, pupicidal activities against malaria vectors. **Chansang *et al.* (2018)** studied the synergistic activity of essential oils of *Cyperus rotundus*, *Alpinia galanga* and *Cinnamomum verum* and permethrin against *Aedes aegypti.*

- **NANOPARTICLES AND MOSQUITO CONTROL**

Different studies have been reported by different workers on the metallic and encapsulated nanoparticles to check their larvicidal activities against different species of mosquitoes. Encapsulation techniques have improved the applications of traditional pesticides and minimized their load on environment. These synthetic pesticides have been encapsulated by several workers.

Paula *et al*. (2011) used the encapsulation of *Lippia sidoides* essential oil with chitosan and angico gum to studied invitro and invivo controlled release, according to their size distribution, polymer ratio and thermal stability. **Santhoshkumar *et al.* (2011)** reported the silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. **Soni *et al*. (2012)** reported gold nanoparticles formed by entomopathogenic fungi *Aspergillus flavus* and checked their ability to the penetrating the cuticle of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. **Forim *et al*. (2013)** studied the polymer encapsulated extract of *Azadirachta indica* to prepare their nanoformulations and reported high entrapment efficiency when compared their UV stability with its commercial products. **Priya and Santhi (2014)** synthesized the silver nanoparticles by using aqueous extract of *Mimosa pudica* against fourth instar larvae of *Anopheles subpictus* and *Culex quinquefasciatus*. **Bhan *et al*. (2014)** evaluated the encapsulated nanoparticles loaded with different ratios of temphos and imidacloprid to study their larvicidal toxicity against *Culex quinquefasciatus*. **Bhan *et al.* (2015)** studied the different encapsulated formulations of synthetic pesticides, temephos and imidacloprid against the larvae of *Anopheles stephensi*. **Murugan *et al.* (2015)** studied the Datura silver nanoparticles activities against malaria vector, *Anopheles stephensi*. **Dinesh *et al.* (2015)** reported silver nanoparticles from *Aloe vera* extract is an effective green tool against the larvae of malaria vector, *Anopheles stephensi*. **Subashini *et al.* (2016)** evaluated the silver nanoparticles leaf extracts of *Azadirachta indica* against larvae of *Culex quinquefasciatus.* **Benelli (2016)** studied the larvicidal activity of plant mediated nanoparticles as an emerging tool against malaria vector, *An. Stephensi*. **Gonzalez *et al.* (2017)** evaluated the properties of two essential oils, *Geranium maculatum*, and *Citrus bergamia* loaded polymeric nanoparticle using polyethylene glycol against *Culex pipiens* larvae. **Adhikari *et al.* (2018)** studied the silver nanoparticles prepared the *swietenia mahagoni* L. against *Anopheles stephensi, Cx. quinquefasciatus* and *Cx. vishuni* larvae.

MORPHO-HISTOLOGICAL STUDIES

➢ MORPHOLOGICAL STUDIES

Khater and Shalaby (2008) studied the biologically active plant of fenugreek, earth almond, mustard and olibanum derived essential oils. some morphological abnormalities in the larvae of *Culex pipens* **Kumar *et al*. (2011)** observed the morphological alterations in *Aedes aegypti* after treated with three species of peppers. **Martins *et al*. (2011)** reported the comparative analysis of the fat body in five different mosquito species (*Aedes albopictus*, *Aedes fluviatilis*, *Culex quinquefasciatus*, *Anopheles aquasalis* and *Anopheles darling*). **Saranya *et al*. (2013)** evaluated the leaf extract of *Spathodea campanulata* effect on *Aedes aegypti* and observed visible morphological deformities such as some pupal melanization (brown colour), dwarfed pupae with retarded abdomen and de-chitinized larvae. **Velu *et al*. (2015)** studied some morphological changes caused by peel extracts of *Arachic hypogaea* in the larvae of *Aedes aegypti* and *Anopheles stephensi*. **Soonwera and Phasomkusolsil (2016)** studied the impact of *Cymbopogon citratus* and *Syzyium aromaticum* essential oils on the morphology against *Aedes aegypti* and *Anopheles dirus* larvae. **Louni *et al*. (2018)** observed the morphology of nanoemulsion was also studied by TEM essential oil *Mentha longifolia* L. and pesticide combination against *Ephestia kuehniella* insecticidal efficacy.

➢ HISTOLOGICAL STUDIES

Corena *et al*. (2004) reported the distribution of carbonic anhydrase (CA) in the midgut of *Aedes aegypti* and inhibition of CA is directly related to lethal consequences for the tested species. **Zayed *et al*. (2009)** evaluated the separation of midgut epithelial cells from the basement membrane, destruction in microvilli and irregular appearance of columnar cells found in treated larvae of *Culex pipens*. **Fallatah *et al*. (2010)** Investigated the botanical extract *Trigonella foenumgraceum* affected the tissues of nerve ganglia, muscles, hind gut and mid gut against *Culex quinquefasciatus* larvae. **Perumalsamy *et al*. (2013)** studied the pellitoriene compound and observed the undistinguished enlarge portions of gastric caeca, alteration

in thorax, midgut and found some debris in the haemolymph of anal gills against third instar treated larvae of *Aedes aegypti*. **Balaji *et al*. (2017)** observed the impregnation onto cotton fabrics repellent bioefficacy of Bulk-DEPA and Nano-DEPA was comparatively investigated against *Culex quinquefasciatus*.

After reviewing the relevant literature, it was observed that no proper attention has been paid on the mosquito larvicidal application of synthetic and plant based polymeric nanoformulations and their joint action. Therefore, plant based polymeric nanoparticles loaded with synthetic pesticide and plant essential oil would be developed for the control of mosquito menace. Further, the morpho-histological impact of the nanoparticles on malaria vector will also be undertaken.

OBJECTIVES

The main aim of the proposed study is to control malaria through managing their vector using polymeric nanoformulations loaded with synthetic pyrethroid and plants essential oil. To achieve this aim following objectives would be undertaken.

- Larvicidal bioefficacy screening of selected synthetic pyrethroids and plant essential oils against *Anopheles stephensi*.
- Joint action of the most effective pyrethroid and essential oil against *An. stephensi* larvae.
- Preparation of polymeric nanoparticles loaded with the most potent combination followed their larvicidal bioefficacy against *An. stephensi*.
- Morpho-histological studies of the target organism treated with potent nanoparticles.

MATERIALS AND METHODS

MATERIALS

- **TARGET ORGANISM:**

The larva of *Anopheles stephensi* is selected for present study.

***Anopheles stephensi* (Liston)**

It is a primary urban vector of malaria in India. Larvae of *A. stephensi* exists in different fresh water habitats such as ponds, streams, marshes, swamps and floats parallel to the water surface. Adults can be distinguished from the other mosquitoes by the presence of discrete blocks of black and white scales on the wings and by the palps which are long as proboscis. Adult males feed on the nectar of flowers, whereas females take blood meals, which help to produce viable eggs. Although, indoor feeding habits have shown no variation between seasons, adult females tend to feed more often at night during the summer months rather than during the day in winter.

- **SYNTHETIC PESTICIDES SELECTED:**

1) **Cypermethrin**

Cypermethrin is a type 2nd synthetic pyrethroid used to control the insect pests. It plays a vital role in agriculture; protection of food stuff, disease vector control and insect pest control **(Abdul-hamid *et al*., 2017)** it behaves as a fast acting neurotoxin in insects. It is easily degraded on soil and plants but can be effective for weeks. It is known to possess relatively low mammalian toxicity; poor dermal absorption, rapid metabolism rate and lack of persistence in the environment and has been larvicidal background against Anopheline mosquito **(Mohan *et al*., 2004).**

2) Deltamethrin

Deltamethrin also belongs to the chemical class pyrethroid based structurally on natural pyrethrin. It is helpful in eliminating and preventing a wide variety of insect. It is primary ingredient used in many commercial household insecticides. It gives quick knockdown effect due to its neurotoxic nature. It is used to control different insect pests of agricultural and medical importance. It is generally considered safe and most powerful synthetic pyrethroid. Deltamethrin has been taken for the present study on the basis of its significant larvicidal background against *Aedes aegypti* and *Anopheles gambiae* **(Norris *et al*. 2015)**.

PLANTS SELECTED:

1) *Jatropha curcas* seeds (Family: Euphorbiaceae)

It is commonly known as physic nut. The plant is commonly found in different regions of South East Asia. This plant shows high agro-industrial potential because the seed produces non-edible oil that can be used as a biofuel. It is a valuable source of medicinally important and has been used for treatment of a wide spectrum of ailments related to skin, cancer, digestive, respiratory and infectious diseases. The seeds of the plant are highly effective for controlling mosquito and can be considered for eco-friendly vector control. It has been selected for proposed work on the basis of its larvicidal background of seeds against mosquito *Aedes aegypti* **(Ojha and Pattabhiramaiah, 2013)**.

2) *Ricinus communis* seeds (Family: Euphorbiaceae)

Ricinus communis is commonly known as castor plant. The plant is widespread through tropical regions and commonly found in jungle areas of India as ornamental plant. It is fast growing suckering perennial shrub or ocassionaly a soft wooded small tree upto 6 mt and not hardy in nature. The seeds are considered as different in size and colour. This plant have various medicinal activites like antifertility, anti-implantation, anticancer, antioxidant, antidiabetic, antiulcer, antimicrobial, antifungal, insecticidal etc.

with no detrimental effects on the body **(Bhakta and Das 2015).** The plant has been selected on the basis of its larvicidal property against *Culex quinquefasciatus* **(Batabyal *et al*., 2009)**.

3) *Cinnamomum tamala* leaves (Family: Lauraceae)

It is commonly known as taizpat in India and naturally found in Asia and Australia. They are evergreen trees and shrubs and most species are aromatic and many are economically important. The essential oil of the leaves called tejpat oil and medically used as carminative, anti-flatulent, diuretic, aromatic, stimulant, antigonorrhea, hypoglycemic, antirheumatic, antidote for scorpion sting and show strong therapeutic effects against cancer and inflammation. The plant bark and leaves are good source of essential oil with significant mosquito larvicidal background against *Culex quinquefasciatus* **(Iqbal *et al*., 2018)** and **(Upadhyay, 2017)**.

METHODOLOGY

- **REARING AND CULTURE OF MOSQUITO:**

The larvae of *Anopheles stephensi* would be collected from natural oviposition sites with standard dipping procedure. Identification of the larvae would be conducted by using the key **(Tyagi *et al*., 2015)**. The larvae would be maintained at 27 ± 2^0 C and 70-80% relative humidity under a photoperiod of 14:10 hours (light/dark) in the laboratory and would be reared in water and feed on fine powdered biscuits and yeast extract in ratio 3:2. Pupae would be transfer from the trays to a cup containing tap water then placed into cages of 23×23×32 cm of diameter. The adults would be reared in the glass cages of 30×30×30 cm dimensions. The adult's colony would be provided with 10% sucrose solution with periodically blood feed on shaving rats. After 3 days of feeding, ovitrap would be kept inside the cages and the eggs would be collected and transfer to the enameled trays at the same conditions. The third instar larvae would be used in bioassay tests. **(WHO, 2005).**

- **EXTRACTION OF ESSENTIAL OILS:**

The seeds of *Jathropha curcas, Ricinus communis* and leaves of *Cinnamomum tamala* would be collected from the field and then washed with running tap water to remove extra impurities and dried in shade at room temperature. The weighed and dried materials of all the plants would separately be subjected to hydrodistillation for 6 h in a Clevenger-type apparatus to get essential oil. Residues obtained above would be dissolved in ethanol to get stock solutions. **(Mohammed *et al*., 2017)**

- **BIOASSAY:**

1) Larvicidal

The essential oils of selected plant materials would be diluted in ethanol to obtain stock solution of the desired strength. The experiments would be set according to WHO standard procedures **(WHO, 2005)**. Twenty, 3rd instar lab acclimatized larvae of *Anopheles stephensi* would be exposed to range of different desired working test concentrations would be prepared by diluting these stocks in water, independently in 250 ml capacity of glass beakers containing 199 ml of tap water and 1ml of test concentration. The experiments would be conducted in triplicates along with control. Mortality count would be made after 24 and 48 hours of post treatment.

2) Joint bioefficacy

Keeping the most effective synthetic pyrethroid as standard, the stock would be mixed with the stock of the most potent essential oil in different ratios and working test concentration for each mixed formulation would be prepared by further diluting the combination in water **(Kalyanasundaram and Das, 1985).** Larvicidal bioassay would be conducted as per above discussed standard protocol.

PREPARATION OF PHYTONANOFORMULATIONS:

Nanoparticles of the most potent combination would be prepared through melt-dispersion method with some modifications using appropriate polymers (synthetic, natural and synthetic-natural) loaded with the most potent combination of synthetic pyrethroid and essential oil. The combination would be mixed in

different quantities with melted polymer & stirred lightly with a glass rod to ensure even distribution of the mixture. The mixture is grounded completely in a mortar box after cooling naturally at 25^0C. Then they are placed in airtight, self-sealable polyethylene pouches & stored at 25^0C in desiccators containing calcium chloride to prevent moisture absorption prior to further use.

MORPHO-HISTOLOGICAL STUDIES:

1) **Morphological study:**

Treated larvae will be studied for morphological alterations under the light microscope. For the observation, larvae would be mounted on the microscopic slide with Hoyer's medium. Morphological changes if any in body segments including head, abdomen, thorax and anal gill would be observed and compared with those of control **(Kumar *et al*., 2011)**.

2) **Histological study:**

The histological studies were performed on the 3rd instar larvae of *An. stephensi*. The larvae were exposed to the corresponding LC_{50} test concentration. Upon the exposure period larvae were isolated and stored in a buffered formalin reagent. Further, the larvae were subjected to dehydration by passing through a graded ethanol series and embedded in the paraffin wax. The longitudinal section of the larval tissues would be cut using a microtome and stained with Hematoxylin and Eosin **(Almehmadi *et al*., 2011)**. The processed sections of the larvae were fixed onto a glass slide and observed under a light microscope. The blocks would be prepared for the treated parts of the gut and sections will cut with a rotatory microtome (5µ-10µ). Deparaffinized the sections and brought down to water through descending series of ethyl alcohol. The section will be stained in Mercuric Bromophenol Blue (MBB) for 15 minutes and following with washed in 0.5% acetic acid for 20 minutes. Further, the sections will immerse in distilled water for 3 minutes and dehydrated in ethanol series 2 dips each of it. Next step will be sectioned tissues in xylene (2 changes) and final is to be mounted in DPX (Di-butyl Phthalate Xylene) and observed under the stereo microscope **(Pearse *et al*., 1975)**.

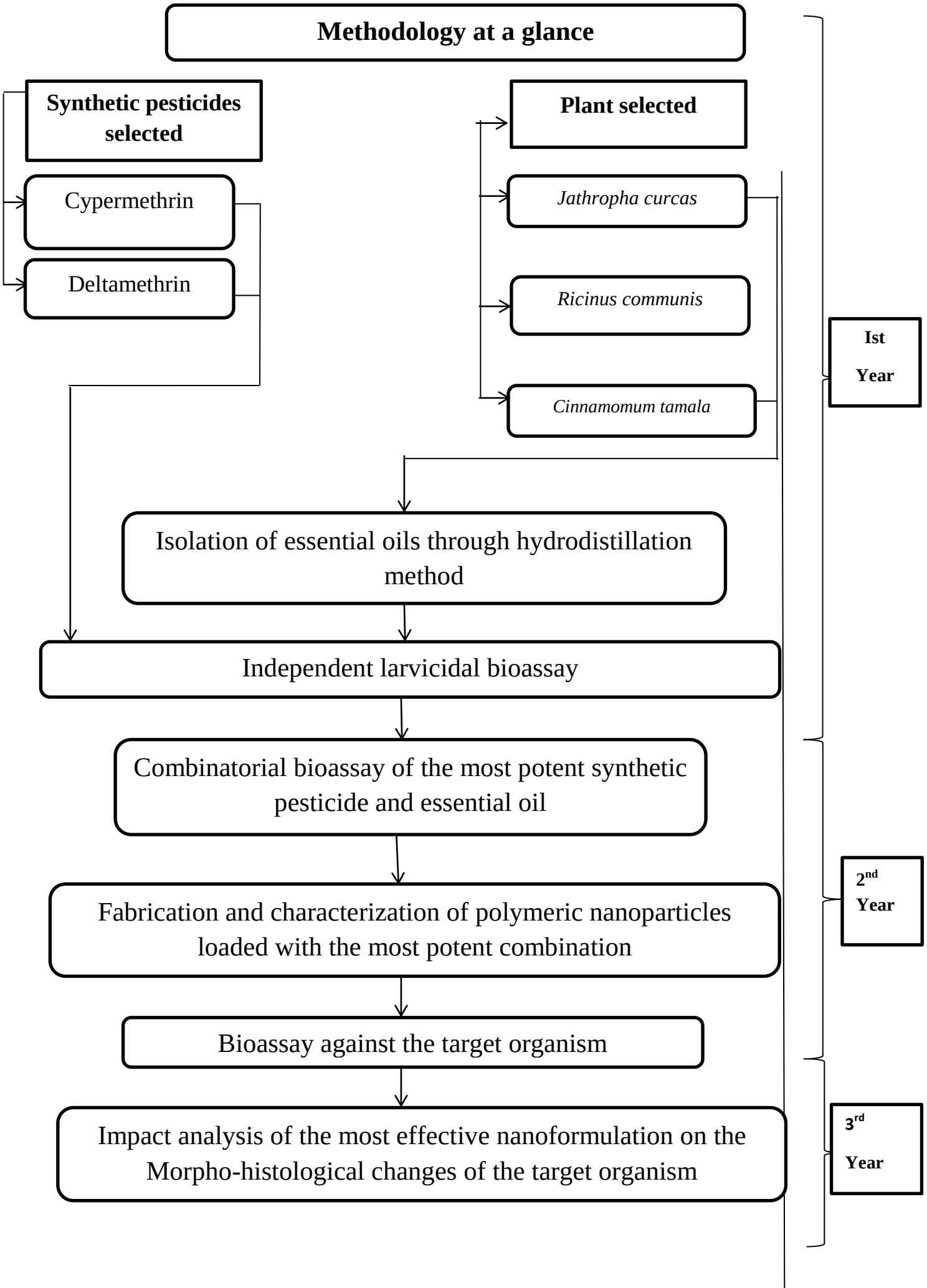
3) DATA ANALYSIS

The observed data would be subjected to probit analysis **(Finney, 1971)** for calculating LC_{50} and LC_{90} and other statistical parameters for all the experiments would be expressed according to the future results**.** When the control mortality ranged from 5-10% the observed mortality percentage would be corrected by using formula **(Abbott, 1925).** Combined factor (CF) for joint efficacy would be calculated using LC_{50} and LC_{90} **(Kalyanasundaram and Das 1985)**.

$$\text{Combined factor (CF)} = \frac{\text{Toxicity of insecticide (alone)}}{\text{Toxicity of insecticide with plant}}$$

CF Value > 1; Indicates synergism and

CF Value < 1; Indicates antagonism


Methodology at a glance
Synthetic pesticides selected
Cypermethrin
Deltamethrin
Plant selected
Jathropha curcas
Ricinus communis
Cinnamomum tamala
Isolation of essential oils through hydrodistillation method
Independent larvicidal bioassay
Combinatorial bioassay of the most potent synthetic pesticide and essential oil
Fabrication and characterization of polymeric nanoparticles loaded with the most potent combination
Bioassay against the target organism
Impact analysis of the most effective nanoformulation on the Morpho-histological changes of the target organism
Ist Year
2nd Year
3rd Year

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